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Maternal and developmental toxicity study of sodium azide in rats

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ABSTRACT

Sodium azide (NaN_3) is being proposed for use as an active ingredient to control a broad spectrum of soil borne pathogens including insects, weeds, nematodes, fungi, and bacteria. The purpose of this study was to determine the maternal and developmental toxicity of NaN_3 in rats. Sperm-positive Sprague–Dawley rats were treated with NaN_3 via oral gavage once daily from Gestation Day (GD) 6 through 19 at respective dose levels of 0, 1, 5, and 17.5 mg/kg/day. From GD 10–12, the high-dose was reduced to 10 mg/kg/day due to maternal mortality. Cesarean section was performed on GD 20 and implantation and resorptions sites, live and dead fetuses were counted. Fetuses were weighed, sexed externally and processed for gross external, visceral and skeletal examinations. A high rate of maternal mortality; reduced gestation body weight, gestation body weight changes and food consumption; decreased corrected body weight and corrected weight gain were observed at 17.5/10 mg/kg/day. Fetal weight was also reduced at 17.5/10 mg/kg/day. There were no maternal deaths, clinical signs or body weight effects that were considered related to NaN_3 at 1 and 5 mg/kg/day. No increase in the incidence of malformations and variations were observed at any of the doses evaluated. Based on the results of this study, the No Observed Adverse Effect Level (NOAEL) and the Lowest Observed Adverse Effect Level (LOAEL) for maternal and developmental toxicity of NaN_3 in rats were considered to be 5 and 17.5/10 mg/kg/day, respectively.

1. Introduction

Sodium azide (NaN_3) has been used for a wide variety of military, laboratory, medical, and commercial purposes. Azide in two forms potassium (KN_3) and sodium (NaN_3) was first used as pesticides in the late 1800s; both were registered by PPG and had limited use in the late 1970s and early 1980s. NaN_3 was as well used as a preservative in aqueous laboratory reagents and biologic fluids and as an ingredient to inflate automobile airbag gas (Chang and Lamm, 2003; Trout et al., 1996). Recent efforts have been made to revitalize the use of NaN_3 as a possible alternative to methyl bromide applications as an insecticide, herbicide, nematocide, fungicide and bactericide. The toxicity of NaN_3 has been extensively studied. NaN_3 a potent vasodilator, potentially causes severe hypotension on accidental exposure (Qamirani et al., 2006). It was found to lower the blood pressure of hypertensive but not normotensive individuals during investigations on the effects of metabolic inhibition on cancer patients (Black et al., 1954). Chang and Lamm (2003) conducted a systematic review of the literature from 1927 to 2002 on human exposure to NaN_3 and its health effects. The most commonly reported health effect from NaN_3 exposure is hypotension. More recent evidence also suggests that the hypotensive side effects of NaN_3 poisoning could be due to the direct vasodilator properties of this compound (Swafford et al., 2005). In addition NaN_3 was shown to inhibit cytochrome oxidase following a 28-day systemic administration in rats of a non-lethal dose via subcutaneous osmotic pumps (Berndt et al., 2001). It binds irreversibly to the heme cofactor in a process similar to carbon monoxide, which is then converted to nitric oxide; it is most likely that this conversion accounts for its toxicity (Smith et al., 1991). Oral administration of the NaN_3 solution in mice produced an increase in locomotor activity for the 12.3 mg/kg group and a decrease for the higher doses (ranging from 16.0 to 27.0 mg/kg). NaN_3 administration suppressed rectal temperature dose-dependently as well as rotarod performance at high-doses (20.8 and 27.0 mg/kg). Such behavioral changes elicited by NaN_3 administration suggest an involvement of the central cholinergic system (Fujimura et al., 2002). Because of its proposed use as a broad spectrum pesticide, there is the potential for possible oral, dermal and inhalation exposures. NaN_3 was mutagenic in Salmonella typhimurium strains TA100 and TA1535, with or without exogenous metabolic activation (S9); it was not mutagenic in strain TA1537 or TA98. There was no evidence of carcinogenic activity of NaN_3 in male or female F344 rats administered 5 or 10 mg/kg (NTP, 1991). Likewise, NaN_3 was found not to be teratogenic in hamsters, and produced embryotoxicity only at dose levels that result in maternal toxicity (Sana et al., 1990). In this study pairs of osmotic

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minipumps containing 400 mg/ml (6.15 M) sodium azide in distilled water were subcutaneously implanted in timed-mated Syrian golden hamsters from GD 7 to GD 9 and animals were euthanized on GD 13. According to our best knowledge there is no literature available on the effect of NaN_3 on embryo-fetal development in rats. Therefore, in this study we have investigated the maternal and developmental toxicity of NaN_3 after exposure during the period of embryonic and fetal periods in rats.

2. Materials and methods

2.1. Experimental design

A total of 114 time-mated CD® [Crl:CD®(SD)] female rats were received from Charles River Laboratories, Portage Michigan. The time-mated females were approximately 8–10 weeks of age at arrival (GD 0). All animals were acclimated from the time of arrival on GD 0 to the time of dosing on GD 6. During this acclimation period, all rats were observed daily for any clinical signs of disease. All animals were given a detailed clinical examination prior to selection on GD 0 and prior to dosing on GD 6.

Prior to assignment to study, the animals were weighed and examined for evidence of disease and other physical abnormalities. Animals assigned to study had body weights within ±20% of the mean body weight. All animals were randomly assigned to study at arrival using a standard, by weight; block randomization procedure based on GD 0 body weights. A total of 100 time-mated female rats (weighing 182–236 g at randomization) were assigned to the treated or control groups. The animals were individually housed in suspended, stainless steel, wire-mesh type cages in an environmentally controlled room. Fluorescent lighting was provided for approximately 12 h per day. Temperature and humidity were monitored and recorded daily. The protocol-designated ranges were 64–79 F and 30–70%, respectively. Basal diet (Lab Diet® Certified Rodent Diet® #5002, PMI Nutrition International, Inc.) was available *ad libitum*.

2.1.1. Dose selection

The doses used in this study were 0, 1, 5, and 17.5 mg/kg/day. The highest dose was selected based on 90-day oral gavage study in rats (NCI, 1981). In this study nearly total mortality occurred at the 20 mg/kg dose over the experimental period, but no deaths occurred at other doses including 10 mg/kg/day. Of the total females in the study, two females died in each week 2, and 6 and six females died on Week 7 of the study. In males the death occurred between weeks 7 and 13 of the study. As the mortality effect observed at 20 mg/kg/day in the NCI (1981) study was over a longer period, and while the duration of the current study was only 14 days; it was decided that a 17.5 mg/kg/day would be appropriate for use as the highest dose in this study.

2.2. Administration

Sodium azide was received from American Pacific and no adjustment was made for purity. Control and test article administration began on GD 6 and continued to include GD 19 for all animals. The test article was administered to the treated groups by oral gavage once per day at approximately the same time each day at dosage levels of 1, 5, and 17.5 mg/kg/day at a dosage volume of 5 mL/kg based on the body weight. From GD 10–12, surviving animals at 17.5 mg/kg/day began receiving a reduced dosage level of 10 mg/kg/day at a dosage volume of 2.86 mL/kg. The control animals received the vehicle, distilled water (buffered at pH 9.5 with sodium hydroxide), at a volume of 5 mL/kg and dosing regimen as the treated animals. Observations of the animals included clinical signs, gestation body weights, and gestation food consumption.

2.2.1. C-section and fetal evaluation

On Gestation Day (GD) 20, each female was euthanized and subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, and live and dead fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and corrected body weight (terminal weight minus uterus weight) and corrected weight gain (final body weight minus uterus weight minus Gestation Day 0 body weight) calculated. All fetuses were weighed, sexed externally, and given a gross external examination. Approximately one-half of the fetuses in each litter were processed for visceral examination, and the remaining fetuses in each litter were double stained with alizarin red and alcian blue for skeletal examination. Malformations and developmental variations were recorded. Fetal observations were recorded using the terminology developed by the International Federation of Teratologists (Wise et al., 1997).

2.3. Statistical procedures

Mean and SDs were calculated for all measured parameters. Gestation body weights, gestation body weight gains, and gestation food consumption were analyzed by ANOVA followed, where appropriate, by Dunnett's test (Dunnett, 1955). Comparison of litter (fetal) body weight data was analyzed by ANOVA; the litter was the unit of observation. For reproductive parameters, a one factor (i.e. treatment group) ANOVA was used for mean corpora lutea, mean total, live and non-live (resorptions and death) implants, mean percent live and non-live implants, and mean percent pre- and post implantation loss. Visceral and skeletal data were analyzed by Fisher's Exact Test. Sex ratio (% male/litter) was transformed using Arcsin square-root transformation. The transformed data was then analyzed using Dunnett's adjusted *t*-test (Dunnett, 1955) and or Welch's *t*-test (Welch, 1937) with a Bonferroni correction as appropriate. A minimum significance level of $p = 0.05$ was used for all comparisons.

3. Results

3.1. Maternal effects

A total of 19 dams in the high-dose group (17.5 mg/kg/day) died and one dam from the same group was euthanized in moribund condition prior to reducing the dose to 10 mg/kg/day. From GD 10–12, the dosage level for the surviving animals ($n = 5$) was then reduced to 10 mg/kg/day. Two out of the five surviving animals died one day and two days, respectively, after the dose was reduced to 10 mg/kg/day. All the animals died between GD 8 and GD 12 of the study. No death was observed in the low- (1 mg/kg/day) and mid-dose group (5 mg/kg/day).

Treatment-related clinical signs of toxicity were observed in the high-dose group (17.5/10 mg/kg/day), which included decreased activity, prostration, loss of righting reflex, lacrimation, impaired limb function, swelling (head and face), moribundity, and difficult/slow/shallow breathing. Although the mortality was NaN_3 induced, no NaN_3-related macroscopic lesions were observed in dams at necropsy.

Gestation body weight and gestation body weight changes were significantly decreased in the animals receiving the high-dose (17.5/10 mg/kg/day; Tables 1 and 2) when compared to the control animals. Gestation body weight and gestation body weight changes were not affected in the animals receiving 1 and 5 mg/